



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Transcriptional regulation by complex interplay between posttranslational modifications

Citation for published version:

Skelly, MJ, Frungillo, L & Spoel, SH 2016, 'Transcriptional regulation by complex interplay between posttranslational modifications', *Current opinion in plant biology*, vol. 33, pp. 126-132.
<https://doi.org/10.1016/j.pbi.2016.07.004>

Digital Object Identifier (DOI):

[10.1016/j.pbi.2016.07.004](https://doi.org/10.1016/j.pbi.2016.07.004)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Current opinion in plant biology

Publisher Rights Statement:

2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons-mons.org/licenses/by/4.0/>)

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.





Transcriptional regulation by complex interplay between post-translational modifications

Michael J Skelly¹, Lucas Frungillo¹ and Steven H Spoel

Transcriptional reprogramming in response to developmental changes or environmental inputs is regulated by a wide variety of transcription factors and cofactors. In plants, the stability of many transcriptional regulators is mediated by the ubiquitin-mediated proteasome. Recent reports suggest that additional post-translational modifications modulate the ubiquitination and thus stability of transcriptional regulators. In addition to well-recognized phosphorylative control, particularly conjugation to the ubiquitin-like protein SUMO as well as thiol modification by nitric oxide to yield S-nitrosothiols, are emerging as key regulatory steps for governing protein ubiquitination in the nucleus. Complex interplay between these different post-translational modifications may provide robust control mechanisms to fine tune developmental and stress-responsive transcriptional programs.

Address

Institute of Molecular Plant Sciences, School of Biological Sciences, University of Edinburgh, King's Buildings, Max Born Crescent, Edinburgh EH9 3BF, United Kingdom

Corresponding author: Spoel, Steven H (steven.spoel@ed.ac.uk)

¹ These authors contributed equally.

Current Opinion in Plant Biology 2016, **33**:126–132

This review comes from a themed issue on **Cell signalling and gene regulation**

Edited by **Kimberley Snowden** and **Dirk Inzé**

<http://dx.doi.org/10.1016/j.pbi.2016.07.004>

1369-5266/© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Introduction

To survive plants must efficiently respond to wide-ranging environmental cues and stresses by rapidly yet precisely reprogramming their transcriptomes. Transcription is regulated by a vast array of transcription factors and associated cofactors that are often subject to diverse post-translational modifications (PTMs). PTMs add a tremendous amount of complexity to cellular proteomes and their large variety and concurrent appearance in transcriptional regulators is thought to dramatically increase the nuclear proteome size from mere thousands to the order of millions of possible protein forms.

Post-translational control of transcriptional regulators by ubiquitination is especially prevalent in plants, with genomic analysis revealing that core components of the ubiquitination machinery may account for up to 6% of the Arabidopsis proteome [1]. Ubiquitination is facilitated by the sequential actions of three enzyme types; E1 ubiquitin-activating enzymes, E2 ubiquitin-conjugating enzymes and E3 ubiquitin ligases. Polyubiquitination of proteins often targets them for degradation by the 26S proteasome, a large multi-protein complex harbouring the major proteolytic activity in all eukaryotic cells. This ubiquitin-proteasome system (UPS) is essential to the regulation of hormone-responsive genes. In some cases hormones are even perceived by co-receptors consisting of both a transcriptional regulator and an E3 ligase component. Hormone binding acts as a molecular glue, promoting recruitment of the transcriptional regulator to the E3 ligase and eventually resulting in its degradation. For example, the hormones auxin and jasmonate promote recruitment of transcriptional repressors to the Skp1/Cullin/F-box (SCF) E3 ligases, SCF^{TIR1} and SCF^{CO11}, respectively, leading to their proteasome-mediated degradation and activation of hormone-responsive gene expression [2,3]. More recently, salicylic acid (SA) was also identified to be perceived by a co-receptor consisting of both the Cullin3-RING E3 ligase (CRL), CRL3^{NPR3/4}, and its substrate NPR1, an indispensable master coactivator of plant immune genes [4–6].

While cross talk between ubiquitination and phosphorylation has been well established [7], interplay between ubiquitination and other PTMs is only just emerging. Here we will highlight emerging evidence of combinatorial regulation of several developmental and stress-responsive transcription (co)factors in plants by ubiquitination and the additional PTMs. The interplay between ubiquitination and other PTMs adds additional layers of complexity to allow plants to fine-tune the nuclear levels and activity of transcriptional regulators.

Combinatorial control by ubiquitination and SUMOylation

In addition to ubiquitin, various ubiquitin-like proteins exist with distinct and diverse functions, including small ubiquitin-like modifier (SUMO). Ubiquitin-like proteins are characterized as such by sharing a similar structure and enzymatic mechanism of conjugation with ubiquitin. Proteomic analyses of SUMO-modified proteins in Arabidopsis have identified hundreds of targets, many of which are involved in transcription regulation [8–10].

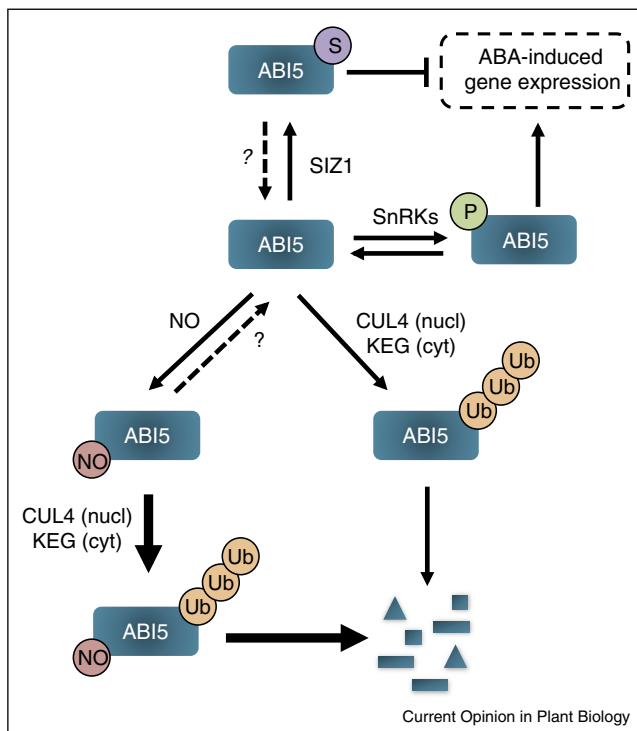
The bZIP transcription factor ABI5 has a central role in abscisic acid (ABA) signalling and is regulated by a multitude of PTMs [11]. ABI5 is phosphorylated by SNF1-related protein kinases (SnRK2.2, SnRK2.3 and SnRK2.6) that promote its transcriptional activity [12] (Figure 1). In absence of ABA, however, ABI5 is maintained at low levels due to ubiquitination by the cytoplasmic E3 ligase KEEP ON GOING (KEG) and subsequently degraded by the 26S proteasome [13,14]. Interestingly, increased levels of ABA promote KEG self-ubiquitination and degradation, leading to stabilization of ABI5 and activation of ABA responses [15]. In contrast to ubiquitination, SUMOylation of ABI5 at Lys391 by the SUMO E3 ligase, SIZ1 (SAP and Miz 1), prevents its degradation [16]. Accordingly, *siz1* mutant plants displayed lower ABI5 protein levels but were curiously

hypersensitive to ABA, suggesting that SUMOylation negatively regulates ABA signalling. Furthermore, blocking SUMOylation of ABI5 by expression of a mutant K391R transgene in *abi5* plants resulted in ABA hypersensitivity. Thus, SUMOylation of ABI5 not only prevents its degradation but also negatively regulates its intrinsic transcriptional activity by an unknown mechanism. Modification of ABI5 by ubiquitin and SUMO appears to occur at different Lys residues (Lys344 and Lys391, respectively) [13,16], suggesting that these two related PTMs do not simply compete for the same site but rather act combinatorially (Figure 1; for effects of S-nitrosylation see discussion below).

In addition to ABI5, the R2R3 MYB-type transcription factor MYB30 mediates ABA signalling and is also SUMOylated by SIZ1 [17]. Similar to ABI5, SUMOylation of MYB30 at Lys283 prevented its degradation and also appeared to be required for its transcriptional activity, as expression of a mutant K283R transgene did not fully restore ABA sensitivity in *myb30* plants. More recently, MYB30-interacting E3 ligase 1 (MIEL1) was identified as a RING-type E3 enzyme responsible for MYB30 ubiquitination and proteasomal degradation [18]. The site(s) of MYB30 ubiquitination are yet to be determined and once revealed may provide further insight into how the UPS and SUMO compete for this substrate.

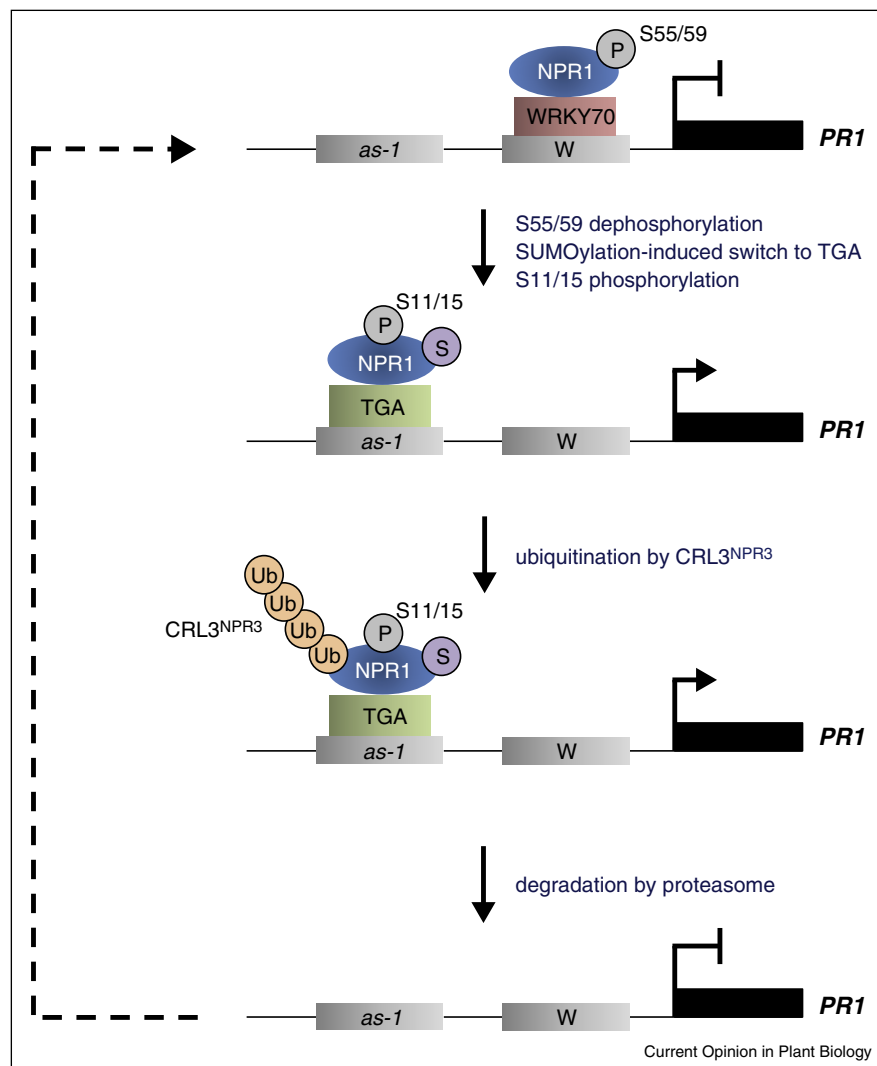
The transcription coactivator NPR1 is a master regulator of SA-responsive genes and associated immunity against biotrophic pathogens. In the absence of SA, nuclear NPR1 is thought to be ubiquitinated by CRL3^{NPR4} and undergoes proteasomal degradation to prevent activation of immune genes [5,19,20]. Immune activation increases SA levels and results in phosphorylation of NPR1 at Ser11/15, probably promoting the switching of NPR1 to the alternate E3 ligase CRL3^{NPR3}. This leads to the ubiquitination and turnover of NPR1 that paradoxically is necessary for full induction of its target genes [19,20]. Recent work has revealed that regulation of NPR1 activity by PTMs is even more complex with the finding that SUMOylation also modulates this coactivator [21]. Modification of NPR1 by SUMO3 appeared to be a prerequisite for phosphorylation at Ser11/15 and was shown to promote its proteasomal degradation. SUMOylation in turn was found to be controlled by a dephosphorylation event at Ser55/59 of NPR1 (Figure 2). Importantly, SUMOylation of NPR1 coactivator was proposed to modulate its association with different transcription factors. While unmodified NPR1 associated with the WRKY70 transcriptional repressor, SUMOylated NPR1 preferentially interacted with the TGA3 transcription activator. Chromatin immunoprecipitation studies of the well-defined SA-responsive *PR1* promoter further showed that mutant NPR1 that cannot be modified by SUMO3 was constitutively localised to a WRKY binding motif known as a W-box motif. By contrast, SA-induced wild-type NPR1 switched its localisation to an *as-1* element that is known to

Figure 1



Multiple post-translational modifications regulate accumulation and activity of the ABA-responsive transcription factor ABI5. The ABA-responsive transcription factor ABI5 is subject to a variety of post-translational modifications that mediate seed germination and plant growth. Phosphorylation (P) by SnRK2 protein kinases (SnRKs) promotes transcriptional activity of ABI5. SUMOylation (S) by the SUMO E3 ligase SIZ1 at K391 stabilizes ABI5 and suppresses its transcriptional activity. Additionally, ABI5 is polyubiquitinated by nuclear (nucl) CUL4 and cytoplasmic (cyt) KEG ubiquitin ligases, and consequently degraded by the proteasome. Nitric oxide strongly promotes ABI5 recruitment to CUL4 and KEG ubiquitin ligases by S-nitrosylating (NO) Cys153 of ABI5. Thus, S-nitrosylation directly or indirectly o

Figure 2



Interplay between phosphorylation, SUMOylation and ubiquitination regulates NPR1 function and activity at the *PR1* gene promoter. In the absence of SA signalling, NPR1 is thought to bind the transcriptional repressor WRKY70 at the W-box element of the *PR1* promoter.

Phosphorylation (P) of NPR1 at S55/59 appears to promote this state by preventing NPR1 SUMOylation. Activation of SA signalling leads to NPR1 dephosphorylation at S55/59, triggering SUMOylation of NPR1 by SUMO3 (S). This SUMOylation allows the switching of NPR1 association from WRKY70 to TGA transcription activators and induction of *PR1* gene expression. Furthermore, SUMOylation of NPR1 promotes its phosphorylation at S11/15, leading to NPR1 ubiquitination (Ub) by the CUL3^{NPR3} E3 ligase and subsequent proteasome-mediated degradation.

be occupied by TGA factors [21^{••}]. Thus, activation of immune genes may require a SUMOylation-induced switch in NPR1 interaction partners (Figure 2). Considering the importance of SUMOylation to ABA signalling, it is interesting to note that CRL3-mediated NPR1 degradation appears to be promoted by ABA, suggesting that hormone cross talk between SA and ABA may be established at the post-translational level by modulation of NPR1 SUMOylation and ubiquitination [22^{••}].

Perception of the hormone gibberellin (GA) also triggers a UPS-mediated signalling pathway. In this case, binding of

GA to its receptor GIBBERELLIN INSENSITIVE DWARF 1 (GID1), promotes association with DELLA transcriptional repressors resulting in recruitment of an SCF^{SLY} E3 ligase complex that targets DELLAs for degradation [23]. In a recent study, DELLAs were shown to be SUMOylated, which not only protects them from degradation, but appears to act as a GID1-sequestering mechanism to allow accumulation of non-SUMOylated DELLAs, thereby limiting plant growth during stress [24]. Indeed, a SUMO-interacting motif (SIM) was identified in GID1 that facilitates this process. Proteins containing SIMs can interact non-covalently with SUMO and

thus SUMOylation can facilitate protein-protein interactions between SIM-containing proteins and SUMO conjugates. Recently, a class of SIM-containing E3 ubiquitin ligases were reported in plants [25] related to the SUMO-targeted ubiquitin ligases (STUbLs) found in yeast and mammals [26,27]. These E3 ligases specifically ubiquitinate SUMOylated proteins. Consequently, SUMO modification of a protein can also result in its ubiquitination and proteasomal degradation. Accordingly, Arabidopsis STUbL4 was shown to reduce protein levels of the transcriptional repressor CYCLING DOF FACTOR 2 (CDF2) to promote flowering, presumably through proteasomal degradation [25]. It is expected that plant STUbLs play various important roles in transcription regulation during hormone signalling due to the prevalence of ubiquitin and SUMO modifications in these pathways.

Protein S-nitrosylation versus ubiquitination

Developmental processes and environmental stress responses often reprogram the transcriptome via alterations in cellular redox potential. Fluctuations in redox potential may be sensed by reactive thiol groups of Cys residues [28]. The diversity of possible thiol redox states offers a molecular framework to use a single residue for a wide range of molecular switches, such as alterations in protein stability, activity, conformation, and localisation. Amongst these different thiol redox states, S-nitrosylation, the addition of a nitric oxide (NO) moiety to a reactive thiol group to form a protein-SNO, has been consolidated as a ubiquitous PTM in plant biology. The past few years have seen many efforts to identify the S-nitrosylated plant proteome and suggest that this PTM plays a key role in many aspects of plant biology [29–33]. While the utilized methodologies often fail to identify specific subcellular or low abundance proteins, several independent reports describe important roles for S-nitrosylation of transcriptional regulators that are also modulated by ubiquitination.

Recently, an intriguing interplay between S-nitrosylation and ubiquitination was demonstrated in the transcriptional control of seed germination by ABA signalling. The ABA-responsive ABI5 transcription factor is a master regulator of seed germination and seedling arrest [34–37]. As described above, the stability of ABI5 is controlled by both SUMOylation and ubiquitination. A new study now suggests that upon seed imbibition a transient burst in NO production leads to S-nitrosylation of ABI5 at Cys153 [38^{••}]. S-nitrosylation of ABI5 did not impact its ability to homo-dimerize nor to bind to its DNA-binding motif, suggesting this modification does not markedly change ABI5 conformation. Instead, S-nitrosylation recruited ABI5 for ubiquitination by both nuclear Cullin4 (CUL4) and cytoplasmic Keep on Going (KEG) E3 ligases, resulting in its degradation by the proteasome (Figure 1). This scenario suggests that SNO modifications evolved to not only regulate protein activity directly, but also by selectively priming proteins for ubiquitin-mediated degradation. Indeed,

S-nitrosylation has been reported to influence a variety of PTMs, including ubiquitination, SUMOylation, phosphorylation, palmitoylation, and acetylation [39]. In this respect it will be important to uncover if and how SNO-induced ubiquitination of ABI5 directly counteracts protective SUMOylation (Figure 1).

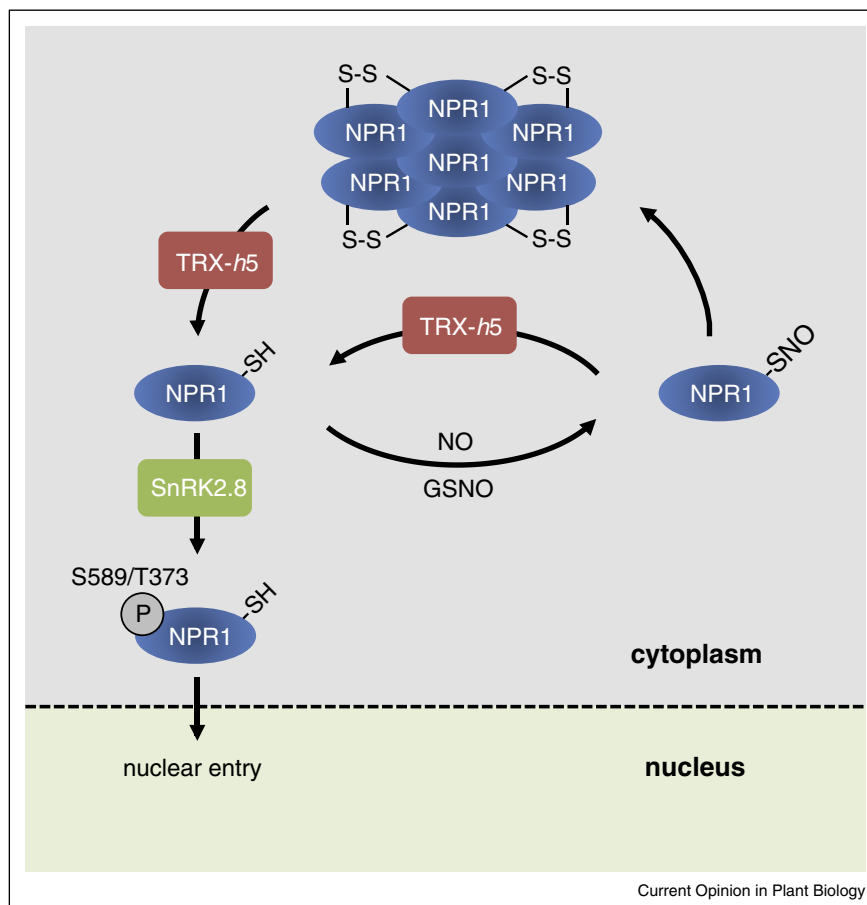
S-nitrosylation has also been reported to regulate the unstable MYB30 transcription factor during the hypersensitive cell death response, an effective strategy to hinder pathogen invasion. MYB30 positively regulates cell death by promoting gene expression and synthesis of very long fatty acids [40]. In unchallenged plants, nuclear levels of MYB30 are kept low by activity of the ubiquitin ligase MIEL1 [18[•]]. Upon infection, expression of MIEL1 is downregulated, thereby raising MYB30 levels and programming cell death. Curiously, MYB30 is also targeted by inhibitory S-nitrosylation at two Cys residues located in its DNA-binding domain [41^{••}]. It is plausible that SNO-induced rejection from the DNA renders MYB30 more susceptible to MIEL1-mediated ubiquitination and degradation. In partial analogy to ABI5, such a mechanism would again put S-nitrosylation at odds with the stabilizing effect of SUMO modifications on MYB30.

The unstable, SA-responsive transcription coactivator NPR1 is subject to several different redox-based modifications. In resting cells, NPR1 is stabilized in the cytoplasm by intermolecular disulphide bonds that generate a large oligomer [42]. Upon activation of immunity, SA induces cellular redox changes that together with the activity of Thioredoxin-*h*5 (TRX*h*5) result in transient reduction of these disulphide bonds, allowing NPR1 monomer to translocate into the nucleus where it activates immune genes. Interestingly, SA is also thought to induce transient S-nitrosylation of NPR1, which facilitates NPR1 re-oligomerization (Figure 3). Because NPR1 exhibits profound instability in the nucleus [19], its transient S-nitrosylation prevents nuclear entry and stabilizes the protein, a process that was shown to be necessary for maintaining NPR1 protein homeostasis during immune responses [43]. Moreover, nuclear entry of NPR1 in sites distal to infection was shown to be mediated by NPR1 phosphorylation at Ser589 and possibly Thr373 by SNF1-RELATED PROTEIN KINASE 2.8 (SnRK2.8) (Figure 3) [44[•]]. Taken together with the fact that the related kinases SnRK2.2 and SnRK2.6 are targeted by inhibitory S-nitrosylation at a Cys residue highly conserved among all members of the SnRK family [45,46[•]], it seems likely that SNOs regulate NPR1 nuclear entry, and thus its stability, at multiple post-translational control points.

Untangling complex PTM networks in transcriptional regulation

In this review we have outlined several recent findings that begin to reveal an increasingly important role for

Figure 3



Regulation of NPR1 nuclear import by redox-based modifications and phosphorylation. In resting cells NPR1 is stabilized in the cytoplasm by formation of large oligomers mediated by intermolecular disulphide bonding (S-S). Transient S-nitrosylation (-SNO) of NPR1 cysteine residues by NO and GSNO stimulates oligomer formation, while their direct reduction by the protein-SNO reductase, TRX-*h5*, promotes the monomeric state. Activation of immunity leads to changes in cellular redox status that together with TRX-*h5* activity reduce NPR1 oligomers to monomers. Monomeric NPR1 is phosphorylated (P) at S589/T373 by SnRK2.8 leading to its nuclear import.

cross-communication between ubiquitination and other PTMs in a wide range of plant developmental and stress response programs. In addition to phosphorylation, particularly SUMOylation and S-nitrosylation are emerging as potent direct and indirect control mechanisms for transcription (co)factor ubiquitination and stability. Identification of enzymes involved in SUMO conjugation and protease pathways is already enabling further functional testing of the various roles SUMOylation may play in transcription-associated ubiquitination events. Research into the enzymatic control of S-nitrosylation is still in its infancy but recent developments indicate that this modification is controlled by at least two SNO scavenging pathways. S-nitrosoglutathione reductase (GSNOR1) controls levels of the physiological NO donor, S-nitrosoglutathione, thereby indirectly regulating the level cellular protein-SNO [47]. By contrast, TRX-*h5* was recently found to act as a direct protein-SNO reductase during

plant immunity and it is likely that other plant TRX enzymes display a similar enzymatic function [48]. GSNOR1 and TRX enzymes are thought to control overlapping but distinct protein-SNO branches and transcriptional programs, so their genetic manipulation has the potential to reveal specific effects of S-nitrosylation on the ubiquitination and SUMOylation of transcriptional regulators. Furthermore, enzymes that generate various post-translational modifications may be modified themselves, thereby introducing an added layer of complexity in the regulation of transcriptional regulators. For example, SnRK2 kinases that phosphorylate and activate ABI5 are themselves targets of inhibitory S-nitrosylation [45,46].

The importance of ubiquitination to plant biology is well illustrated by the fact that many pathogen effectors modulate the activity of host E3 ligases and in some cases even mimic them to promote virulence [49,50].

Similarly, cases are now being uncovered in which effectors act on different PTMs that cross talk with ubiquitin. For example, the type III effector XopD from *Xanthomonas euvesicatoria* displays deSUMOylation activity towards the ethylene-responsive transcription factor SIERF4 from tomato. XopD-mediated deSUMOylation caused destabilization of SIERF4 and suppressed its transcriptional activity likely through ubiquitin-mediated degradation [51^{••},52]. Hence, SUMOylation, S-nitrosylation and most probably other PTMs are emerging as unexpected but integral regulators of ubiquitin signalling in the plant nucleus.

Acknowledgements

We apologize to colleagues whose work we did not cite due to space limitations. This work was funded by a fellowship from the European Molecular Biology Organization (EMBO-ALTF 420-2015) co-funded by the European Commission (LTFCONFUND2013, GA-2013-609409) to LF and grants to SHS from The Royal Society (UF140600), the Biotechnology and Biological Sciences Research Council (BB/L006219/1), and a European Research Council Starting Grant (678511).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Vierstra RD: **The ubiquitin-26S proteasome system at the nexus of plant biology.** *Nat Rev Mol Cell Biol* 2009, **10**:385-397.
 2. Santner A, Estelle M: **Recent advances and emerging trends in plant hormone signalling.** *Nature* 2009, **459**:1071-1078.
 3. Kelley DR, Estelle M: **Ubiquitin-mediated control of plant hormone signaling.** *Plant Physiol* 2012, **160**:47-55.
 4. Manohar M, Tian M, Moreau M, Park SW, Choi HW, Fei Z, Friso G, Asif M, Manosalva P, von Dahl CC *et al.*: **Identification of multiple salicylic acid-binding proteins using two high throughput screens.** *Front Plant Sci* 2014, **5**:777.
 5. Fu ZQ, Yan S, Saleh A, Wang W, Ruble J, Oka N, Mohan R, Spoel SH, Tada Y, Zheng N *et al.*: **NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants.** *Nature* 2012, **486**:228-232.
 6. Wu Y, Zhang D, Chu JY, Boyle P, Wang Y, Brindle ID, De Luca V, Despres C: **The Arabidopsis NPR1 protein is a receptor for the plant defense hormone salicylic acid.** *Cell Rep* 2012, **1**:639-647.
 7. Hunter T: **The age of crosstalk: phosphorylation, ubiquitination, and beyond.** *Mol Cell* 2007, **28**:730-738.
 8. Budhiraja R, Hermkes R, Müller S, Schmidt J, Colby T, Panigrahi K, Coupland G, Bachmair A: **Substrates related to chromatin and to RNA-dependent processes are modified by Arabidopsis SUMO isoforms that differ in a conserved residue with influence on desumoylation.** *Plant Physiol* 2009, **149**:1529-1540.
 9. Miller MJ, Barrett-Wilt GA, Hua Z, Vierstra RD: **Proteomic analyses identify a diverse array of nuclear processes affected by small ubiquitin-like modifier conjugation in Arabidopsis.** *Proc Natl Acad Sci U S A* 2010, **107**:16512-16517.
 10. Elrouby N, Coupland G: **Proteome-wide screens for small ubiquitin-like modifier (SUMO) substrates identify Arabidopsis proteins implicated in diverse biological processes.** *Proc Natl Acad Sci U S A* 2010, **107**:17415-17420.
 11. Yu F, Wu Y, Xie Q: **Precise protein post-translational modifications modulate ABI5 activity.** *Trends Plant Sci* 2015, **20**:569-575.
 12. Nakashima K, Fujita Y, Kanamori N, Katagiri T, Umezawa T, Kidokoro S, Maruyama K, Yoshida T, Ishiyama K, Kobayashi M *et al.*: **Three Arabidopsis SnRK2 protein kinases, SRK2D/SnRK2.2, SRK2E/SnRK2.6/OST1 and SRK2I/SnRK2.3, involved in ABA signaling are essential for the control of seed development and dormancy.** *Plant Cell Physiol* 2009, **50**:1345-1363.
 13. Liu H, Stone SL: **Cytoplasmic degradation of the Arabidopsis transcription factor abscisic acid insensitive 5 is mediated by the RING-type E3 ligase KEEPER ON GOING.** *J Biol Chem* 2013, **288**:20267-20279.
- Direct interactions were demonstrated between ABI5 and the E3 ligase KEG. Proteasomal degradation of ABI5 mediated by KEG was shown to require Lys344 of ABI5, suggesting this residue is likely the site of ubiquitination. Degradation of ABI5 was shown to occur in the cytoplasm and thus may exist as a mechanism to prevent nuclear accumulation of ABI5 and subsequent ABA signalling.
14. Stone SL, Williams LA, Farmer LM, Vierstra RD, Callis J: **KEEP ON GOING, a RING E3 ligase essential for Arabidopsis growth and development, is involved in abscisic acid signaling.** *Plant Cell* 2006, **18**:3415-3428.
 15. Liu H, Stone SL: **Absciscic acid increases Arabidopsis ABI5 transcription factor levels by promoting KEG E3 ligase self-ubiquitination and proteasomal degradation.** *Plant Cell* 2010, **22**:2630-2641.
 16. Miura K, Lee J, Jin JB, Yoo CY, Miura T, Hasegawa PM: **Sumoylation of ABI5 by the Arabidopsis SUMO E3 ligase SIZ1 negatively regulates abscisic acid signaling.** *Proc Natl Acad Sci U S A* 2009, **106**:5418-5423.
 17. Zheng Y, Schumaker KS, Guo Y: **Sumoylation of transcription factor MYB30 by the small ubiquitin-like modifier E3 ligase SIZ1 mediates abscisic acid response in Arabidopsis thaliana.** *Proc Natl Acad Sci U S A* 2012, **109**:12822-12827.
- MYB30 was identified in a mutant screen for genes involved in ABA signalling and SIZ1 was shown to SUMOylate this transcription factor at Lys283 in planta. Loss of SIZ1 function resulted in degradation of MYB30 in response to ABA, suggesting SUMOylation stabilizes MYB30. Furthermore, constitutive expression of wild-type, but not the K283R mutant of MYB30 rescued ABA hypersensitive phenotypes of myb30 mutant plants. This suggests that SUMOylation is also required for full transcriptional activity of MYB30.
18. Marino D, Froidure S, Canonne J, Ben Khaled S, Khafif M, Pouzet C, Jauneau A, Roby D, Rivas S: **Arabidopsis ubiquitin ligase MIEL1 mediates degradation of the transcription factor MYB30 weakening plant defence.** *Nat Commun* 2013, **4**:1476.
- A yeast two-hybrid screen identified an E3 ligase, MIEL1 as a MYB30-interacting protein. MYB30 was shown to be ubiquitinated by MIEL1 and degraded by the 26S proteasome. Accordingly, loss of MIEL1 function resulted in increased expression of MYB30 target genes.
19. Spoel SH, Mou Z, Tada Y, Spivey NW, Genschik P, Dong X: **Proteasome-mediated turnover of the transcription coactivator NPR1 plays dual roles in regulating plant immunity.** *Cell* 2009, **137**:860-872.
 20. Furniss JJ, Spoel SH: **CULLIN-RING ubiquitin ligases in salicylic acid-mediated plant immune signaling.** *Front Plant Sci* 2015, **6**:154.
 21. Saleh A, Withers J, Mohan R, Marqués J, Gu Y, Yan S, Zavaliev R, Nomoto M, Tada Y, Dong X: **Posttranslational modifications of the master transcriptional regulator NPR1 enable dynamic but tight control of plant immune responses.** *Cell Host Microbe* 2015, **18**:169-182.
- SUMOylation of NPR1 by SUMO3 was shown to switch its association from repressor WRKY proteins to TGA activators at target gene promoters. NPR1 SUMOylation was shown to be a prerequisite for its transcription-associated proteasomal degradation and development of SA-dependent immunity. Furthermore, new sites of NPR1 phosphorylation were revealed that inhibit its SUMOylation.
22. Ding Y, Dommel M, Mou Z: **Absciscic acid promotes proteasome-mediated degradation of the transcription coactivator NPR1 in Arabidopsis thaliana.** *Plant J* 2016, **86**:20-34.
- Using exogenous chemical treatments combined with ABA-deficient and ABA-accumulating mutants, it was shown that ABA promotes NPR1 degradation mediated by CRL^{NPR3/4} ligases. SA-induced phosphorylation of NPR1 might prevent its ABA-mediated degradation, suggesting a novel mechanism by which hormone cross-talk fine tunes NPR1-mediated transcriptional responses in plant immunity.
23. Sun TP: **The molecular mechanism and evolution of the GA-GID1-DELLA signaling module in plants.** *Curr Biol* 2011, **21**:R338-R345.

24. Conti L, Nelis S, Zhang C, Woodcock A, Swarup R, Galbiati M, Tonelli C, Napier R, Hedden P, Bennett M *et al.*: **Small ubiquitin-like modifier protein SUMO enables plants to control growth independently of the phytohormone gibberellin.** *Dev Cell* 2014, **28**:102-110.
25. Elrouby N, Bonequi MV, Porri A, Coupland G: **Identification of Arabidopsis SUMO-interacting proteins that regulate chromatin activity and developmental transitions.** *Proc Natl Acad Sci U S A* 2013, **110**:19956-19961.
26. Elrouby N: **Extent and significance of non-covalent SUMO interactions in plant development.** *Plant Signal Behav* 2014, **9**:e27948.
27. Sriramachandran AM, Dohmen RJ: **SUMO-targeted ubiquitin ligases.** *Biochim Biophys Acta* 2014, **1843**:75-85.
28. Spoel SH, Loake GJ: **Redox-based protein modifications: the missing link in plant immune signalling.** *Curr Opin Plant Biol* 2011, **14**:358-364.
29. Lindermayr C, Saalbach G, Durner J: **Proteomic identification of S-nitrosylated proteins in Arabidopsis.** *Plant Physiol* 2005, **137**:921-930.
30. Romero-Puertas MC, Campostriani N, Matte A, Righetti PG, Perazzolli M, Zolla L, Roepstorff P, Delledonne M: **Proteomic analysis of S-nitrosylated proteins in Arabidopsis thaliana undergoing hypersensitive response.** *Proteomics* 2008, **8**:1459-1469.
31. Camejo D, Romero-Puertas Mdel C, Rodriguez-Serrano M, Sandalio LM, Lazaro JJ, Jimenez A, Sevilla F: **Salinity-induced changes in S-nitrosylation of pea mitochondrial proteins.** *J Proteomics* 2013, **79**:87-99.
32. Hu J, Huang X, Chen L, Sun X, Lu C, Zhang L, Wang Y, Zuo J: **Site-specific nitrosoproteomic identification of endogenously S-nitrosylated proteins in Arabidopsis.** *Plant Physiol* 2015, **167**:1731-1746.
33. Chaki M, Shekariesfahlan A, Ageeva A, Mengel A, von Toerne C, Durner J, Lindermayr C: **Identification of nuclear target proteins for S-nitrosylation in pathogen-treated Arabidopsis thaliana cell cultures.** *Plant Sci* 2015, **238**:115-126.
34. Antoni R, Rodriguez L, Gonzalez-Guzman M, Pizzio GA, Rodriguez PL: **News on ABA transport, protein degradation, and ABFs/WRKYs in ABA signaling.** *Curr Opin Plant Biol* 2011, **14**:547-553.
35. Finkelstein RR, Lynch TJ: **The Arabidopsis abscisic acid response gene ABI5 encodes a basic leucine zipper transcription factor.** *Plant Cell* 2000, **12**:599-609.
36. Lopez-Molina L, Chua NH: **A null mutation in a bZIP factor confers ABA-insensitivity in Arabidopsis thaliana.** *Plant Cell Physiol* 2000, **41**:541-547.
37. Lopez-Molina L, Mongrand S, Chua NH: **A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in Arabidopsis.** *Proc Natl Acad Sci U S A* 2001, **98**:4782-4787.
38. Albertos P, Romero-Puertas MC, Tatematsu K, Mateos I, Sánchez-Vicente I, Nambara E, Lorenzo O: **S-nitrosylation triggers ABI5 degradation to promote seed germination and seedling growth.** *Nat Commun* 2015, **6**:8669.
- Genetic screens for Arabidopsis seed germination upon pharmacological treatment with ABA and NO led to identification of the ABA-insensitive and NO-insensitive *abi5* mutant. By applying the biotin-switch technique the authors show that ABI5 is S-nitrosylated at Cys153. S-nitrosylation of ABI5 did not impact its DNA-binding capacity but promoted its recruitment to CUL4-based and KEG ubiquitin ligases, resulting in its proteasome-mediated degradation. The authors conclude that ABI5 plays a pivotal role in controlling seed germination by mediating cross talk between ABA and NO.
39. Hess DT, Stamler JS: **Regulation by S-nitrosylation of protein post-translational modification.** *J Biol Chem* 2012, **287**:4411-4418.
40. Raffaele S, Vaillau F, Léger A, Joubès J, Miersch O, Huard C, Blée E, Mongrand S, Domergue F, Roby D: **A MYB transcription factor regulates very-long-chain fatty acid biosynthesis for activation of the hypersensitive cell death response in Arabidopsis.** *Plant Cell* 2008, **20**:752-767.
41. Tavares CP, Vernal J, Delena RA, Lamattina L, Cassia R, Terenzi H: **S-nitrosylation influences the structure and DNA binding activity of AtMYB30 transcription factor from Arabidopsis thaliana.** *Biochim Biophys Acta* 2014, **1844**:810-817.
- The DNA-binding activity of the Arabidopsis MYB30 transcription factor is shown to be impaired by incubation with the NO donor SNP. The biotin-switch assay was applied to show that two Cys residues present in the MYB30 DNA-binding domain are targets of S-nitrosylation *in vitro*. By using circular dichroism, the authors show that reduced DNA-binding activity of MYB30 was due to S-nitrosothiol-induced alterations in its secondary structure.
42. Mou Z, Fan W, Dong X: **Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes.** *Cell* 2003, **113**:935-944.
43. Tada Y, Spoel SH, Pajerowska-Mukhtar K, Mou Z, Song J, Wang C, Zuo J, Dong X: **Plant immunity requires conformational changes of NPR1 via S-nitrosylation and thioredoxins.** *Science* 2008, **321**:952-956.
44. Lee HJ, Park YJ, Seo PJ, Kim JH, Sim HJ, Kim SG, Park CM: **Systemic immunity requires SnRK2.8-mediated nuclear import of NPR1 in Arabidopsis.** *Plant Cell* 2015, **27**:3425-3438.
- Gene expression analysis revealed a strong induction of the *SnRK2.8* gene, encoding a SNF1-related kinase, in distal tissues of Arabidopsis plants after inoculation with avirulent *P. syringae*. Genetic evidence indicated that SnRK2.8 is required for induction of SA-dependent systemic acquired resistance. Notably, this kinase was shown to interact and phosphorylate the immune coactivator NPR1 at sites distal to attempted infection. SnRK2.8-mediated phosphorylation of NPR1 was necessary for its SA-induced translocation into the nucleus where it activates immune genes.
45. Wang P, Du Y, Hou YJ, Zhao Y, Hsu CC, Yuan F, Zhu X, Tao WA, Song CP, Zhu JK: **Nitric oxide negatively regulates abscisic acid signaling in guard cells by S-nitrosylation of OST1.** *Proc Natl Acad Sci U S A* 2015, **112**:613-618.
46. Wang P, Zhu JK, Lang Z: **Nitric oxide suppresses the inhibitory effect of abscisic acid on seed germination by S-nitrosylation of SnRK2 proteins.** *Plant Signal Behav* 2015, **10**:e1031939.
- Amino acid sequence alignment showed that the primary structure of all 10 members of the SnRK family in Arabidopsis share strong amino acid similarities. Notably, residue Cys137, known to be modified by inhibitory S-nitrosylation in SnRK2.6 is conserved among all proteins examined. In addition to SnRK2.6, *in vitro* pharmacological assays indicated that SnRK2.2 is also reversibly inhibited by S-nitrosylation. Given the amino acid sequence similarity among the member of the SnRK family member, the authors argue that kinase activity of other members of the SnRK family is also regulated through S-nitrosylation.
47. Feechan A, Kwon E, Yun BW, Wang Y, Pallas JA, Loake GJ: **A central role for S-nitrosothiols in plant disease resistance.** *Proc Natl Acad Sci U S A* 2005, **102**:8054-8059.
48. Kneeshaw S, Gelineau S, Tada Y, Loake GJ, Spoel SH: **Selective protein denitrosylation activity of thioredoxin-h5 modulates plant immunity.** *Mol Cell* 2014, **56**:153-162.
49. Duplan V, Rivas S: **E3 ubiquitin-ligases and their target proteins during the regulation of plant innate immunity.** *Front Plant Sci* 2014, **5**:42.
50. Marino D, Peeters N, Rivas S: **Ubiquitination during plant immune signaling.** *Plant Physiol* 2012, **160**:15-27.
51. Kim JG, Stork W, Mudgett MB: **Xanthomonas type III effector XopD desumoylates tomato transcription factor SIERF4 to suppress ethylene responses and promote pathogen growth.** *Cell Host Microbe* 2013, **13**:143-154.
- The type III secretion effector XopD from *Xanthomonas euvesicatoria* (Xcv), which causes bacterial spot of tomato, contains a SUMO protease domain. Here the authors report that XopD directly targets the ethylene-responsive transcription factor SIERF4 of tomato. XopD probably deSUMOylated Lys53 of SIERF4, which was associated with reduced SIERF4 stability and immune-related transcriptional output. Thus, the data in this report suggest that SUMO modifications of transcriptional regulators may be an important hijacking point for successful plant pathogens.
52. Kim JG, Taylor KW, Hotson A, Keegan M, Schmelz EA, Mudgett MB: **XopD SUMO protease affects host transcription, promotes pathogen growth, and delays symptom development in xanthomonas-infected tomato leaves.** *Plant Cell* 2008, **20**:1915-1929.